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# Effect of *Azadiracta indica* Leaves Extract on Fungi Causing Black Rot and Scabs in Apple Fruit

Ejimofor Chiamaka Frances[1], Oledibe Odira Johnson[2*]
[1]Department of Biological Sciences, Chukwuemeka Odumegwu University, Uli, Anambra State, Nigeria.
[2] Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
*Corresponding author

**Abstract:- Black rot and scab diseases of apple are caused by fungus and they are highly economic important disease to the farmers. It reduces the production and the quality of apple at both pre and post-harvest period. This research was conducted to ascertain the antifungal effect of *Azadiracta indica* leaves extract on diseased apple fruit. The samples (apple fruit) were analyzed and examined using standard laboratory procedures. Ethanol was used in extraction of *Azadiracta indica* (neem) leaf while chloramphenicol was used as control. The result of the analysis showed fungual pathogen such as *Aspergillus spp, Fusarium spp* and *Venturia inaequalis* were found to cause rot and scab on apple fruit. The result also showed that ethanol extract of *Azadiracta indica* leaf extract tend to have more inhibitory effect on these organisms. The highest inhibitory effect by *Azadiracta indica* (neem) leaf extract showed on *Venturia inaequalis* (15. 8mm) and lowest on *Aspergillus spp* (11.2 mm) at 6mg/ml while the highest good inhibitory effect by chloramphenicol on *Venturia inaequalis* was 9.1mm and lowest on *Aspergillus spp* was 6.2mm. This showed that the neem leaf extract have antifungal properties that can be helpful in the control and management of scab fungi of apple fruit. Therefore, this study recommended that neem extract should be adopted by farmers in the control of black rot and scab fungi in apple, as this method of control is much safe and efficient.**

***Keywords:-*** *Fungi, Black rot, Venturia inaequalis.*

## I. INTRODUCTION

Fungi are eukaryote that absorbs nutrients directly through its cell walls and digests food externally. They reproduce by spores and their body is composed of tubular cells called hyphae. They are heterotrophs and, like animals, obtain their carbon and energy from other organisms. [1].

Neem (*Azadirachta indica* A. Juss.) is a medium-sized tree, reaching 15 to 30 m in height, with a large rounded crown up to 10-20 m in diameter. It is a multipurpose tree that is highly popular in India, where it provides food and insecticide, and is used for its great number of ethno medicinal properties. It is mainly evergreen but sometimes shed its leaves during the dry season [2].

The neem is a tropical evergreen plant. It has shown great importance in the research because of potential of using neem derivatives such as leaf, oil and seed extracts for preparation of environmental friendly herbicides. Leaf extract of neem can inhibit the aflatoxin production as well as *Aspergillus* parasiticus growth. [3]. Neem leaves are used to treat fungal and bacterial infections. They are used to treat warts as well as chicken pox, either the paste is applied on the affected area or the person is made to bath in neem water, it can also treat foot fungi [4, 5].

The Antifungal effects of neem leaf extract also reported from south America against *Crinipellis perniciosa* and *Phytophthora* species causing Witches broom and Pot Not of cocoa [6].

An apple is a sweet edible fruit produced by an apple tree (*malus domestica*). Apple trees are cultivated world-wide and are the most widely grown species in the genus malus [7].

Fruits and juice produced from apple are used as foods because its dependable source of vitamins, minerals, electrolytes, antioxidants and fiber. The fruits are usually eaten fresh and raw except for the seeds making the nutritional values fully available for the body. The increasing understanding of the link between fruit intake and improved health coupled with the newly found nutritional values of apple (*malus domestica*) has increased its popularity and thus consumption rate. [8, 9, 10, 11, 12].

Apples are rich in phytochemicals which have been reported to reduce risk of cardiovascular diseases, asthma; diabetes, cataracts, Alzheimer's disease/cognitive decline and pulmonary functions [8,13, 14, 15, 16,12].

Apple trees and fruit are prone to a number of fungal, bacterial and pest problems which can be controlled by a number of organic and non-organic means. The black rot is an important disease of apple caused by the fungus *Botryosphaeria obtuse*. Black rot fungus infects a wide variety of hardwood trees, including apple and pear. Apple scab or black rot is also caused by the fungus *venturia inaequalis* which occurs almost everywhere apples are grown and is the most serious and widespread disease of this crop, especially in region with high rainfall and relative humidity during the growing season [17].

However, fungi cause most plant disease, accounting for perhaps 70% of all major crop diseases [18, 19]. Apart from the effects of high temperature and relative humidity, fungi produce pectic enzymes that break down apple pectin to expose the nutrients of the cells to the fungi [20, 21].

The high levels of sugars and nutrients content in apple, maks them desirable for fungal growth [22]. These Fungi can penetrate apples, particularly through a puncture or other wound that breaks the skin of the apple. Toxigenic fungi have been isolated from spoiling fruits [23].

Fruits stored at suboptimal conditions promotes fungal growth and mycotoxin production [24] of which the most common causes of apple rot are from the fungi *Penicillium expansum* and *Monilinia fructigena* [25, 26]. Other fungal genera that were isolated from apples include *Colletotrichum*, *Xylaria*, *Botryosphaeria* and *Rhizopus* oryzae [27, 28]. *Aspergillus* spp. has also been isolated and known to cause infections or allergies [29]. In some studies, *Cladosporium* spp. was found to be a frequent fungus found in stored apples, and also *Penicillium*, *Acremonium*, *Aspergillus*, *Aureobasidium*, *Cryptococcus*, *Sporobolomyces* and *Alternaria* spp [30].

Traditional practices for studies of fungi include conventional cultivation and microscopic identification. Identification of the fungi species is based on mycelia (color, size and shape) and morphological characteristics (morphology, conidia size and morphology conidiophore) [31, 32]. These techniques require skilled taxonomists. Minor differences in medium composition can impair effective comparison of mycelia characters [33]. Molecular techniques have been demonstrated as an effective and easy way to identify fungi. DNA-based assays are reliable to detect a variety of fungi. Various molecular approaches have been used for the detection of *Aspergillus* from environmental and clinical samples [34].

Considering the above facts, this study has been undertaken to analyze the antifungal properties of neem extracts against some pathogenic fungi causing the black rot and scabs in apple fruits in Nigeria.

## II. MATERIALS AND METHOD

Apple fruit (*Malus domestica*) was purchased from Unizik Junction and transported in a sterile bag to Maeve academic research laboratory where it was identified before being analyzed in the laboratory.

### 2.1 Experimental Equipment

The equipment's used in the experiment includes, autoclave, knife, binocular microscope, microscopic slide, slid cover, conical flask, 250ml and 500ml beaker, inoculating loop, cotton wool, cork borer, foil, burnson burner petri dish and masking tape.

### 2.2 Experimental Reagents

The reagents used includes Distilled water, Ethanol, lacto phenol, Potato Dextrox agar (PDA).

### 2.3 Samples Preparation Procedure

**Preparation of *Malus domestica* (Apple fruit)**

The surface of the sample *Malus domestica* (Apple fruit) was first surface sterilized with 70% alcohol and the infected portion cut off into small pieces of size 5cm by 5cm which weighing 1grams then kept in beaker ready for use.

The Neem plant (*Azardiracta indica*) were dried under room temperature at 25± $1^0$c for 5days. The sample was grinded into powdery form and ready for analysis.

**Extraction of Neem plant (*Azardiracta indica*)**

The aqueous neem leaf extract was prepared according to the method described by [35]. 100g of the plant sample was weighed and into a Soxhlet extractor were it was extracted using ethanol as extraction solvent.

### 2.4 Culture Media Preparation

In the study, the culture medium assay used was Potato Dextrose Agar (PDA). This medium was used for the growth and maintenance of the isolated fungal. The Potato Dextrose Agar (PDA) was prepared according to the manufacturer instruction (39 g in 1l of water). The medium was sterilized in the autoclaved at 121 °C and 15 psi for 20 min for complete dissolution and homogeneity. Thereafter, it was allowed to cool to temperature of between 42 and 45 °C. A capsule of chloramphenicol was added to every 500 ml of sterile cooled PDA to prevent bacteria growth [36]. 15 ml of the cooled amended PDA was poured into each sterile petri dish to solidify. The petri dishes that contained the medium were incubated for 24h at a temperature of 28 °C to check for sterility before use as described by Cheesebrough [37].

### 2.5 Colony Count

The direct colony count method was used. In this method, the colonies of fungi were counted directly from the cultured plate.

### 2.6 Identification of Fungi Isolate

Both morphological and anatomical characteristics of the fungi were used in the identification process of the fungi isolate. This was done as follows.

**A: Morphological identification**

Methods of as described by Cheesebrough [37]: The growth pattern and pigments produced by the fungi was observed, match against those in fungi identification kit and recorded accordingly.

**B: Anatomical Identification:**

Here, guides according to Alexopoulos [38] were used for the various tests and examinations. A smear of the fungi growth was fix on two different slide and stained with distil water and lacto phenol. Both of the smears were viewed under binocular microscope and the anatomical

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characteristic recorded and as well match against those on fungi identification kit then identified accordingly.

### 2.7 Pathogenicity Test

The method of Okafor [39], Okigbo and Ikediugwu [40] were adopted for the pathogenicity study to establish which of the fungal isolates caused the rot and to determine whether they could induce similar symptoms on inoculation and be re-isolated, thus fulfilling Koch's postulates.

The fungal culture was isolated using inoculation loop of length 5cm while a healthy apple fruit was surface sterilized using 100% ethanol. With the use of cork borer, a smooth deep cut was made on the healthy yam tuber to a depth of 2cm with a diameter of 10mm wide. The pure culture of the fungi was inoculated and the 10mm cover of the initial cut healthy apple fruit part was used to cover it. The sample was kept in an incubation chamber for 72hr then being observed every 3 days to ascertain the pathogenicity of the organism.

### 2.8 Anti-microbial Assay

The food poisoning techniques was used in studying the effect of plant extract on mycelia growth of the test fungi [41]. 4mg/l, 6mg/l and 8mg/l of the plant extract were pipetted into labelled petri dishes containing the pure culture of the microbial organism respectively. The Inhibition zones of the microorganism was evaluated and recorded in terms of radial growth of the microbes on the medium with and without extracts and results were analysed on the basis of percentage growth zone inhibitions of microorganisms. The inhibition of microbial growth on PDA medium was used to quantify the toxicity of extracts. Percentage growth inhibition for 5 days was calculated.

$$\% \text{ growth inhibition} = \frac{R_1 - R_2}{R_2} X \quad 100$$

Where $R_1$ = is the furthest radial distance of pathogens in control plates

Where $R_2$ = is the furthest radial distance of pathogens in extract (treated) plates. The percentage of growth inhibition was determined as a guide in selecting the minimum inhibition concentration that will be effective in controlling the microorganisms.

### 2.9 Statistical Analysis

The two-way analysis of variance (ANOVA) were used in analyzing the data collected, using the Sigma plot version 12 statistical software to ascertain the level of significance of the treatment given to at $LSD_{0.05\%}$.

## III. RESULTS

**Table 3.1 Anti-Microbial Inhibition zone of *Azadiracta indica* Extract at different dose concentration**

| Microorganism | 2mg/ml) | 4mg/ml | 6mg/ml | Control (Chloramphenicol) |
|---|---|---|---|---|
| *Aspergilius spp* | 8.10mm | 9.73mm | 11.2 mm | 6.2mm |
| *Fusarrium spp* | 9.20mm | 9.17mm | 13.35 mm | 6.8mm |
| *Venturia inaequalis* | 5.6mm | 10.0mm | 15. 8mm | 9.1mm |
| | | | | |

Table 3.1 shows that at 2mg/ml concentration, the extract inhibition on A*spergilius spp* fungi is 8.10mm, on *Fusarium spp* it si 9.20mm while *Venturia inaequalis* is 5.6mm. At this dosage concentration of 2mg/ml, the inhibition of *Apergilius spp* and *Fusarium* is high as compared to that of *Venturia inaequalis* while inhibition on *Fusarium spp* is higher than that of *Aspergilius spp*. At 4mg/l the level of inhibition on *Aspergilius spp* is 9.73mm, 9.17mm on *Fusarium spp* and 10mm on *Venturia inaequalis.* The inhibition of the extract at 6mg/ml on *Aspergilius spp* is 11.2mm, that of *Fusarium spp* is 13.35mm while on *Venturia inaequalis*, the inhibition rate is 15.8mm. Comparing the level of inhibition of the neem extract with that of the control experiment as seen in the table above, the performance of the extracts is much better than the control. This implies that neem extract is far better in the control of apple fungi.

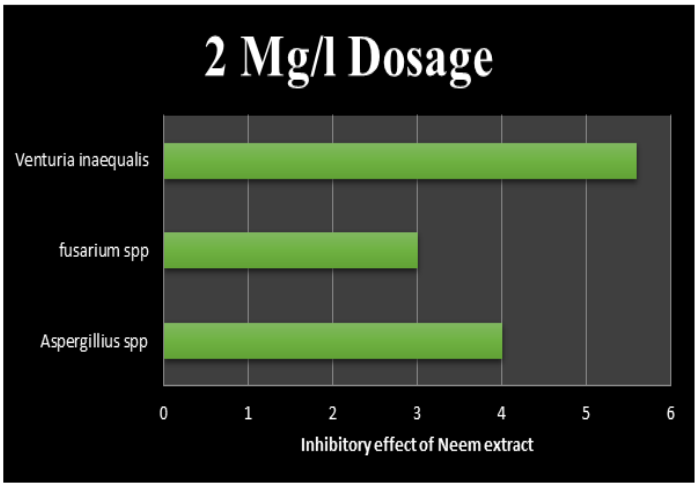


**Fig 1: Inhibition of microorganism at 2mg/l**

Fig 1 shows that at 2mg/l treatment dosage *Fusarium spp* is highly inhibited by the extract followed by that *Aspergilius spp* and least in *Venturia inaequalis.*

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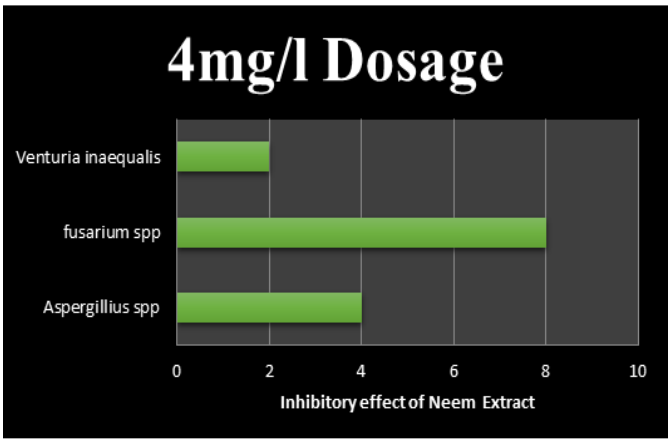


**Fig 2: Inhibition of microorganism at 4mg/l**

Fig 2: at 4mg/l treatment dosage, *Venturia inaequalis* is highly inhibited by the extract followed by that of *Aspergilius spp* and least in *fusarium spp*

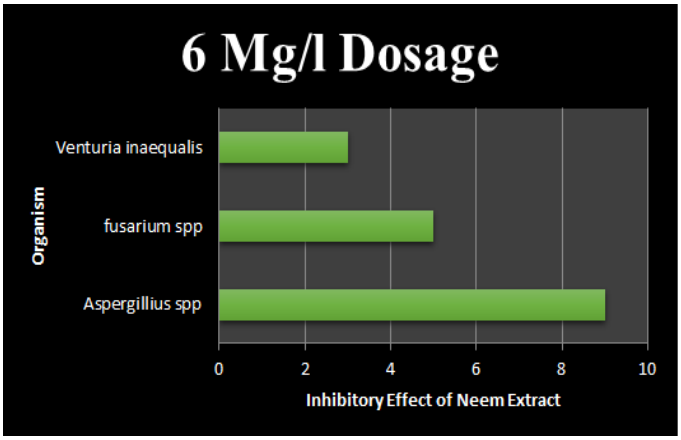


**Fig 3: Inhibition of microorganism at 6mg/l**

Fig 3 shows that at 6mg/l treatment dosage, *Venturia inaequalis* is highly inhibited followed by that of *Fusarium spp* and least in *Aspergilius spp*

**Table 3.2: Two-way ANOVA**

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 1 | 6.000 | 6.000 | 3.350 | 0.141 |
| Residual | 4 | 7.165 | 1.791 | | |
| Total | 5 | 13.165 | | | |

**Tested at 0.05% level of Significance**

Table 3.2 shows that there is no significant differences between the treatment groups at P<0.05(P =0.141), which implies that even at very low dosage of the treatment, it has effect on the fungi associated with the spoilage of apple fruit.

## IV. DISCUSSION

Finding of the study showed that there are various fungi organisms which can cause scab on apple fruit. Such fungi organisms are *Venturia inaequalis, Fusarium spp* and *Aspergilius spp.* These organisms were also found to be inhibited by neem extract at various concentration. The inhibition of these organisms varies from different concentrations ranging from 2mg/l, 4mg/l and 6mg/l respectively. This means that fungi that causes scab on apple fruit can easily be controlled using neem leaf extracts. These findings agree with the finding of [42] who evaluated the efficacy of various extracts of neem leaf on seed borne fungi *Aspergillus* and *Rhizopus* and still confirmed that the growth of the *Aspergillus and Rhizopus* species were significantly inhibited and controlled with both water and alcoholic extract.

The finding also showed that the dosage treatment of these organism is independent of the concentration of the neem dosage which implies that at any dosage treatment, the organisms will be inhibited. The finding also corroborate with that of Anjali [43], who in his study showed that *Azadirachta indica* inhibits growth in *Cladosporium, Aspergillus flavus* and *Alternaria solani*. The study equally agrees with that of Ghonmode [44] who said that there are significant zones of inhibition by the leaf extracts of neem than 3% Sodium hypochlorite.

## V. CONCLUSION

This study has shown that ethanol extract of *Azadiracta indica* leaf has inhibitory effect on the fungi that causes scab on apple fruits. The extract as well is independent of the concentration given as a low concentration also has the capacity of limiting and controlling the growth of the fungi.

## RECOMMENDATION

Based on the finding of this study, it is recommended that the use of Neem extract should be adopted by farmers in the control of apple scab fungi, as this method of control is much safe and efficient.

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